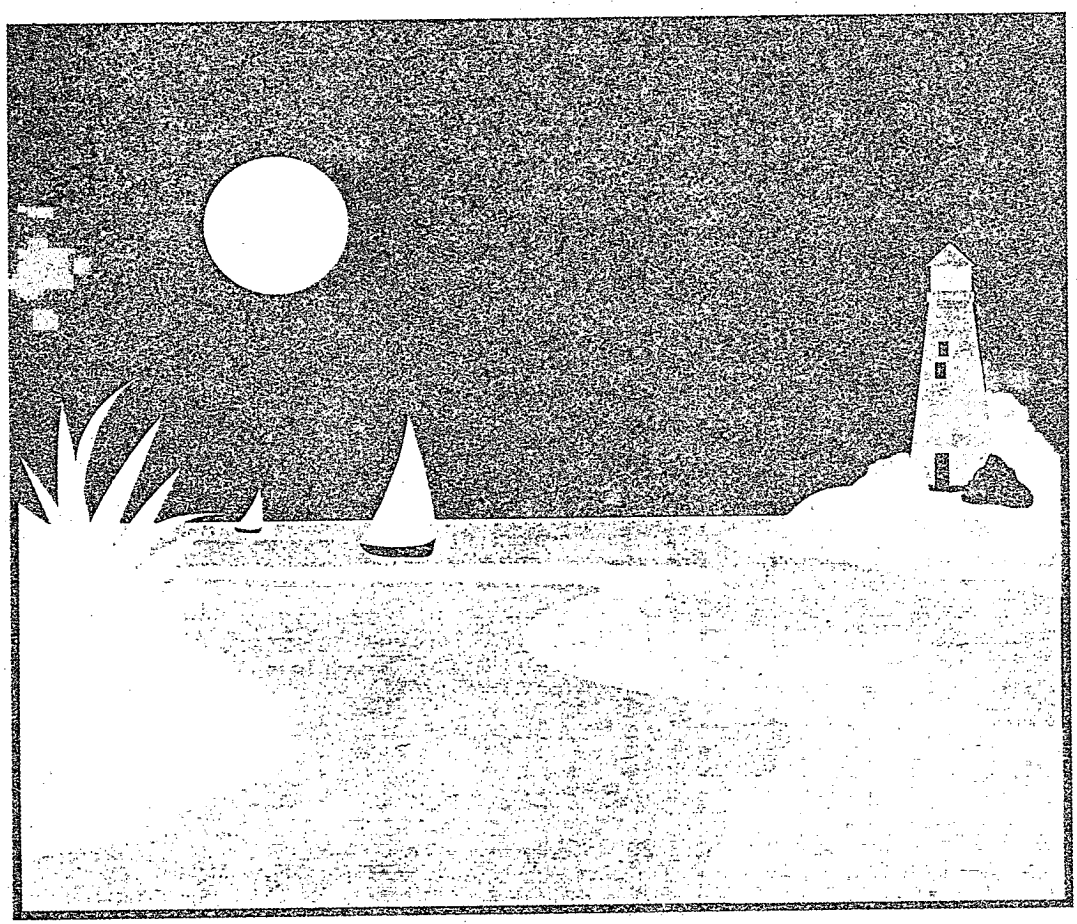
PROGRAM & ABSTRACTS

1996

Summer Neuropeptide Conference



June 23-27, 1996
Martha's Vineyard, Massachusetts, USA

1996 SUMMER NEUROPEPTIDE CONFERENCE

JUNE 23 - 27, 1996

MARTHA'S VINEYARD, MASSACHUSETTS, USA

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The Summer Neuropeptide Conference is an Affiliate of The International Neuropeptide Society

1996 Summer Neuropeptide Conference

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1996 Summer Neuropeptide Conference

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1996 Summer Neuropeptide Conference

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ABSTRACT FORM
1996 Summer Neuropeptide Conference

Please include title, authors, and affiliations. This form is for both symposia and posters. The blank form may be photocopied for additional abstract submissions. Please type in camera-ready format. The book of abstracts will be distributed at the meeting.
Dimensions of Abstract: 20 cm x 12 cm
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NEUROPEPTIDE Y AND ITS RECEPTORS IN THE REGULATION OF REPRODUCTIVE HORMONE SECRETION. Jon E. Levine, Janice H. Urban, Angela C. Bauer-Dantoin, Leslie M. Besecke, and Sarah L. Leupen. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Neuropeptide Y (NPY) is released at several levels of the reproductive axis to regulate female reproductive cyclicity. In the hypothalamus, NPY regulates release of luteinizing hormone-releasing hormone (LHRH) into the hypophysial portal vasculature; at anterior pituitary gland, NPY exerts pronounced modulatory effects on LHRH-stimulated LH secretion. In a series of studies, we have examined the integral role that NPY secretion may play at these loci in the production of mid-cycle gonadotropin surges. Using *in situ* hybridization of NPY mRNA in hypothalamus, we have documented a clear-cut increase in NPY gene expression that is specifically associated with the elaboration of LH surges in proestrus rats. That NPY release is augmented in concert with this increased NPY expression has been confirmed. In addition to an acute increase in NPY expression in anticipation of LH surges, we have also characterized an acute up-regulation of both hypothalamic (LHRH release) and pituitary (LHRH-induced LH release) responsiveness to the facilitory effects of NPY. Pharmacological analysis revealed that a Y1 or Y1-like subtype mediates NPY's actions. We therefore performed quantitative RT-PCR assays of Y1 receptor mRNA throughout the estrous cycle of female rats, to determine if increased NPY receptor expression mediates increased tissue responsiveness to the peptide. While NPY Y1 receptor mRNA fluctuated with a clear ovulatory cyclicity, the expression of the receptor gene was acutely increased after the gonadotropin surge, rather than before; we have thus proposed that preovulatory increases in pituitary responsiveness to NPY are manifest by post-receptor events, or by unmasking of cryptic receptors. Further RT-PCR experiments have suggested that the increase in Y1 receptor gene expression following the gonadotropin surge is dependent upon neural signals for the surge, viz. NPY secretion on proestrus. It is proposed that signalling via Y1 receptors is up-regulated prior to the elaboration of the surge, and that the major stimulus for receptor gene expression is occupancy and activation of NPY receptors by the peptide. Rebound receptor gene expression may serve as a compensatory mechansim which replenishes receptor precursers for mobilization during the subsequent ovulatory cycle. A primary role of NPY and its receptors during the ovulatory cycle may be to amplify activity at several levels within the reproductive axis, and thereby ensure efficacious signal transfer between hypothalamus, pituitary, and gonads. The amplification of these events by NPY may function to permit robust hormone secretions under adaptive physiological conditions, viz. those in which acceptable metabolic, feeding, photoperiodic conditions prevail.

Special audiovisual needs other than a slide projector? NO

Mail to: Dr. Jacqueline Crawley, National Institute of Mental Health, Building 10 Room 4N212, Bethesda, MD 20892-1380 USA
Deadline date is: April 5, 1996

ABSTRACT FORM
1996 Summer Neuropeptide Conference

Please include title, authors, and affiliations. This form is for both symposia and posters. The blank form may be photocopied for additional abstract submissions. Please type in camera-ready format. The book of abstracts will be distributed at the meeting.
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Neurotransmitter and Neuropeptide Alterations in Alzheimer's Disease.

Vahram Haroutunian, Steven Gabriel and Kenneth L. Davis. Department of Psychiatry, The Mount Sinai School of Medicine, New York, NY and the Bronx VA Medical Center, Bronx, NY.

Cerebrocortical deficits in the levels of a variety of neuropeptides are among the characteristic features of Alzheimer's disease (AD). These deficits, though pronounced, are neither uniform across regions of the cerebral cortex, nor evident for all neuropeptides. Because neuropeptides are localized to specific cerebrocortical cell types, and because they are often co-localized with specific neurotransmitter systems, patterns of neuropeptide deficits can provide insight regarding the vulnerability of specific neuronal populations to the disease process. Comparison of patterns of neuropeptide deficits in AD with neuropeptide deficits in aged schizophrenics suggests that different neuropeptides, and by extension different cell groups, are differentially affected in the two diseases. In elderly schizophrenics the pattern of frontal cortical neuropeptides deficits is SLI > NPY > VIP $\geq$ CCK $\geq$ CRH, whereas in the same region of the cerebral cortex the pattern of neuropeptide deficits is CRH $\geq$ SLI > CCK > VIP = NPY. Analysis of neuropeptide deficits in schizophrenics suggests that the calbindin-immunoreactive, kainic acid / AMPA but not NMDA sensitive, SLI, NPY, and SLI+NPY co-localizing GABAergic neurons of superficial layers of the cortex are particularly vulnerable to the disease process. In contrast, the neuropeptide deficits pattern in AD implicates the vulnerability of those SLI localizing neurons which do not also co-localize NPY. This differential vulnerability of SLI vs. NPY vs. SLI+NPY co-localizing hypothesis is supported by the generally greater reduction of SLI in schizophrenics relative to AD cases. Additional support for this hypothesis can be gleaned from the known co-localization of NPY with NOS / NADPHd+ neurons which do not appear to degenerate in AD. Despite the profound reductions in the levels of these different neuropeptides in cognitively impaired AD and schizophrenic cases, the levels of none of the neuropeptides examined in any of the cortical region studied correlated significantly with the magnitude of the cognitive deficits or with the magnitude of neuropathological lesions such as the density of neuritic plaques and neurofibrillary tangles. In contrast to the neuropeptides, the profound deficits in cortical cholinergic markers are relatively uniform across the different cortical regions sampled from AD cases and correlate significantly with the magnitude of cognitive impairment and the density of neuropathological markers. These latter findings suggest that if neuropeptide deficits are associated with any of the symptoms of AD, they are more likely to be associated with non-cognitive behavioral impairments than with the magnitude of dementia.

Special audiovisual needs other than a slide projector?_____

Mail to: Dr. Jacqueline Crawley, National Institute of Mental Health, Building 10 Room 4N212, Bethesda, MD 20892-1380 USA
Deadline date is: **April 5, 1996**

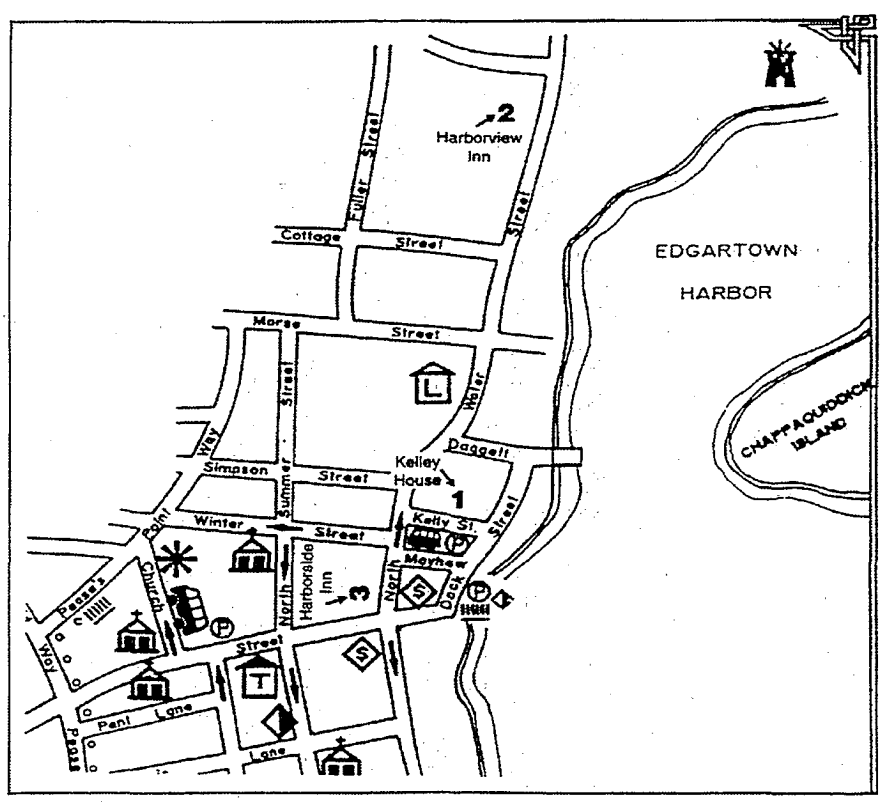
CONFERENCE AND HOTEL LOCATIONS

Harborview Hotel, *131 North Water Street, Edgartown*

Registration, Sunday , June 23rd, 10 AM to 3:30 PM, Lobby outside Menemsha Room (contact conference organizers for registration at other times)
Keynote Lecture and Reception, Sunday 4:00 PM to 6:30 PM, Menemsha Room
All Symposia, Monday through Thursday, 8:30 AM, Tuesday 4:30 PM, Menemsha Room
Poster Session, Monday 5:00 to 7:00 PM, Edgartown Room
Conference Banquet, Wednesday 7:00 PM, Edgartown Room

Kelley House, *23 Kelly Street (no conference events)*

Colonial Inn, *38 North Water Street (no conference events*



GRADUATE STUDENT AND POSTDOCTORAL FELLOW AWARDS

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School of Psychology
University of Ottawa
Ottawa, Ontario, CANADA

Ms. Judith McIntosh
School of Psychology
University of Ottawa
Ottawa, Ontario, CANADA

PRELIMINARY PROGRAM

1996 SUMMER NEUROPEPTIDE CONFERENCE

June 23-27th, Martha's Vineyard, MA, USA

Sunday, June 23rd

4:00pm WELCOME
Jacqueline Crawley and Stafford McLean, Conference Co-Organizers

4:15 KEYNOTE LECTURE
Jeffrey M. Friedman, M.D., Ph.D.
Rockefeller University, New York, NY
Lipostasis and the Control of Body Weight

5:15 **OPENING RECEPTION**

Monday, June 24th

AM SYMPOSIUM: **Neuropeptide Y: Preclinical Advances**
Chair: Markus Heilig

8:30 Markus Heilig, Karolinska Hospital, Stockholm, Sweden
Functional Role of Neuropeptide Y in the Central Nervous System

9:00 Mary Walker, Synaptic Pharmaceutical Corporation, Paramus, NJ
The Y-Type Receptor Family: Cloned Receptors for Neuropeptide Y, Peptide YY, and Pancreatic Polypeptide

9:30 Donald Gehlert, Lilly Research Laboratories, Indianapolis, IN
Recent Advances in the Molecular Pharmacology of NPY Receptors: The Search for the Appetite Receptor

10:00 *Coffee Break*

10:30 Alejandro Daniels, Glaxo Wellcome Inc., Research Triangle Park, NC
Functional Antagonism of Neuropeptide Y Receptors

11:00 Claudine Serradeil-Le Gal, Sanofi Recherche, Toulouse France
The First Generation of Orally-Active NPY Y_1 Antagonists: Pharmacological Profile of SR 120819A

11:30 Yvan Dumont, McGill University, Montreal, Canada
Neuropeptide Y Receptor Subtypes in Mammalian Brain: Evidence for a BIBP 3226 Insensitive Binding Site Labeled with ^{125}I-Leu^{31},Pro^{34}-PYY

5:00-7:00pm **WINE AND CHEESE POSTER SESSION**

Tuesday, June 25th

AM SYMPOSIUM: **Neuropeptides and Mechanisms of Drug and Alcohol Dependence**
Chair: Friedbert Weiss

8:30 Larry Grupp, University of Toronto, Canada
Regulation of Alcohol Intake by Angiotensin Mechanisms

9:00 Friedbert Weiss, Scripps Research Institute, La Jolla, CA
Role of CRF in Drug and Alcohol Withdrawal Syndromes

9:30 Zoltan Sarnyai, Rockefeller University, New York, NY
Role of Oxytocin in the Neuroadaptation to Psychostimulant Drugs

10:00 *Coffee Break*

10:30 David Malin, University of Houston, Houston, TX
Role of Neuropeptide FF in Opiate Tolerance and Dependence

11:00 Glen Hanson, University of Utah, Salt Lake City, UT
Effects of Psychostimulant Drugs on Neurotensin Systems in Limbic Regions

PM SYMPOSIUM: **Limbic-Hypothalamic Peptides in Reproductive Endocrinology**
Chair: Louis Muglia

4:30 Donald Pfaff, Rockefeller University, New York, NY
Neuropeptide Modulation of Reproductive Behavior

5:00 Jon Levine, Northwestern University, Evanston, IL
Neuropeptide Y and its Receptors in the Regulation of Reproductive Hormone Secretion

5:30 William Rostene, INSERM U 339, Paris, France
Neurotensin Effects in Hypothalamic Neuroendocrine Systems

6:00 Paul Micevych, University of California, Los Angeles, CA
Opiate-CCK Interaction in Limbic-Hypothalamic Circuits Regulating Reproductive Behaviors

6:30 Louis Muglia, Harvard Medical School, Boston, MA
Targeted Mutation of Neuropeptides Regulating Reproduction in the Mouse

Wednesday, June 26th

AM SYMPOSIUM: **Mechanisms Mediating the Neurotrophic Actions of Neuropeptides**
Chair: Richard Zigmond

8:30 Richard Zigmond, Case Western Reserve University, Cleveland, OH
Mechanisms of Regulation of Neuropeptide Expression After Neuronal Injury

9:00 Ray Hill, Merck Sharp and Dohme Research Laboratories, Harlow, England
Acidic Fibroblast Growth Factor Stimulates Axonal Regeneration

9:30 Walter Lichtensteiger, University of Zurich, Switzerland
Stage- and Region-Specific Expression of Different Melanocortin Receptor Types in Developing Central and Peripheral Nervous System: Are they Functional?

10:00 *Coffee Break*

10:30 Kim Seroogy, University of Kentucky, Lexington, KY
Neurotrophic Factors and their Receptors in Central Catecholamine Systems: Expression and Regulation

11:00 Doug Brenneman, National Institute of Child Health and Human Development, Bethesda, MD
Vasoactive Intestinal Peptide Increases Neuronal Survival Through an Astroglia-Derived Stress Protein

11:30 Jeff Kordower, Rush Presbyterian-St. Luke's Medical Center, Chicago, IL
Grafts of Trophic Factor Secreting Cells in Rodents and NonHuman Primate Models of Neurodegenerative Disease

7:00pm **CONFERENCE BANQUET**
Announcement of Graduate Student Fellow Awards

Thursday, June 27th

AM SYMPOSIUM: **Neuropeptides as Cognitive Enhancers in the Aged Brain: Hopes and Realities**
Chair: Remi Quirion

8:30 Vahram Haroutunian, Mount Sinai School of Medicine, Bronx, NY
Neurotransmitter and Neuropeptide Alterations in Alzheimer's Disease

9:00 Remi Quirion, Douglas Hospital, McGill University, Montreal, Canada
Neurotensin as a Modulator of Altered Cholinergic Functions in the Brain: Relevance to Neurodegenerative Diseases

9:30 Jacques Epelbaum, INSERM, Paris, France
Somatostatin: Alterations in Neurodegenerative Diseases and Potential Usefulness in the Clinics

10:00 *Coffee Break*

10:30 Pierrette Gaudreau, McGill University, Montreal, Canada
Growth Hormone-Releasing Factor and its Receptors in Aging: Relevance to Altered HPA Functions and Metabolism

11:00 David de Wied, Rudolph Magnus Institute, Utrecht, The Netherlands
Promises and Limitations of Neuropeptides as Cognitive Enhancers

11:30 Illana Gozes, Tel Aviv University, Israel
Neuroprotective Strategy for Alzheimer's Disease: Intranasal Administration of Neuropeptides

12:00 **Round-Up and Final Discussion**

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SYMPOSIUM ABSTRACTS

LIPOSTASIS AND THE CONTROL OF BODY WEIGHT, J. M. Friedman, M.D., Ph.D., The Rockefeller University/Howard Hughes Medical Institute.

Mutations in the mouse ob and db genes result in obesity and diabetes in a syndrome resembling morbid human obesity. Coleman, using the method of parabiosis, predicted that the ob gene encoded a novel hormone and that the db gene encoded its receptor. Recent data from this laboratory are consistent with this hypothesis. The ob gene was identified by positional cloning and found to encode a 4.5 kB RNA expressed exclusively in adipocytes. The ob gene product, known as LEPTIN, circulates as a 16 kilodalton protein in mouse and human plasma but is undetectable in plasma from C57BL/6J ob/ob mice. Plasma levels of this protein are increased in diabetic (db) mice, a mutant thought to be resistant to the effects of ob. The levels of protein are also increased in several other genetic and environmentally induced forms of rodent obesity including mice with lesions in the hypothalamus. Daily intraperitoneal injections of recombinant mouse leptin reduced body weight of ob/ob mice by 30% at 2 weeks and by 40% after four weeks but had no effect in db/ob mice. The protein reduced food intake and increased energy expenditure in ob/ob mice. Injections of wild type mice twice daily with the mouse protein resulted in a sustained 12% weight loss, decreased food intake and a reduction of body fat from 12.2 to 0.7%. Recombinant human leptin reduced body weight with equivalent potency to mouse leptin when injected into ob mice. In human, the plasma level of leptin correlated with body mass index (BMI) and % body fat 18. However, at a given BMI, there was significant variability in the leptin level. In all cases analyzed weight loss in human was associated with a decrease in plasma leptin concentration. These data suggest that leptin serves an endocrine function to regulate body fat stores. In most instances, obesity is associated with an apparent decrease in sensitivity to endogenous leptin resulting in a compensatory increase in adipocyte mass. However, in a subset of cases human obesity appears to result from subnormal leptin secretion.
The complete insensitivity of db mice to leptin and the identical phenotype of ob and db mice suggested that the db locus encodes the leptin receptor. The db gene was localized to a 300 kB interval on mouse chromosome 4. Exon trapping and cDNA selection identified a candidate gene in this region. This candidate was found to be identical to a receptor (ob-R) which was functionally cloned from choroid plexus. However, because this receptor was normal in db mice, the possibility was raised that the db mutation affected an alternatively spliced form. The Ob-R gene was found to encode at least five alternatively spliced forms. One of the splice variants is expressed at a high level in the hypothalamus and at a lower level in other tissues. This transcript is mutant in C57BL/Ks db/db mice. The mutation is the result of abnormal splicing leading to a 106 bp insertion into the 3' end of its RNA. The mutant protein is missing the cytoplasmic region and is likely to be defective in signal transduction. A nonsense mutation in facp rats, a rat equivalent of db, leads to premature termination NH2-terminal of the transmembrane domain (unpublished data). These data suggest that the weight reducing effects of leptin are mediated by signal transduction through a receptor in the hypothalamus and elsewhere.

VASOACTIVE INTESTINAL PEPTIDE INCREASES NEURONAL SURVIVAL THROUGH AN ASTROGLIA-DERIVED STRESS PROTEIN, D. E. Brenneman and I. Gozes, Section on Developmental and Molecular Pharmacology, NICHD, NIH, Bethesda, MD; Department of Clinical Biochemistry, Sackler School o f Medicine, Tel Aviv University, Tel Aviv, Israel.

Neuronal survival during development and aging is determined by a complex series of cellular interactions involving electrical activity as well as limiting amounts of target and glia-derived trophic substances. A neurotrophic protein has been purified from conditioned medium of rat cerebral cortical astroglia stimulated by vasoactive intestinal peptide. Sequential chromatographic separations by ion exchange, gel permeation and hydrophobic interaction were utilized to obtain about 1650-fold purification of a single, 14,000 Dalton protein (apparent pI: 8.3 ± 0.25) that increased survival (EC50, 0.075 pg/ml) of electrically blocked spinal cord neurons, and accordingly it was named: activity dependent neurotrophic factor (ADNF). Amino acid sequence analysis of proteolytic digests indicated homology to a portion of heat shock protein 60 (HSP60). Neutralizing antiserum to HSP60 produced a 40% decrease in neuronal survival, suggesting an endogenous HSP-60-like molecule that is important for the survival of some cerebral cortical neurons. Treatment of cultures with recombinant HSP60 has no detectable effect on neuronal survival. Based on these analyses, a 14 amino acid peptide was synthesized that exhibited an identical EC50 to intact ADNF in a neuronal survival assay. The peptide had a similar but not identical amino acid composition as a fragment of HSP60. This is the first demonstration of a neurotrophic factor whose activity was mimicked by a synthesized peptide and whose neuro-protective activity was evident at femtomolar concentrations. This peptide prevented neuronal cell death associated with the envelope protein (glycoprotein 120) from the human immunodeficiency virus, with excitotoxicity (N-methyl D-asparate), with the beta amyloid peptide (putative cytotoxin in Alzheimer's disease) and with tetrodotoxin (electrical blockade). These studies identify a potent neuroprotective glial protein and an active peptide that provide a basis for developing treatments of currently intractable neurodegenerative diseases.

FUNCTIONAL ANTAGONISM OF NEUROPEPTIDE Y RECEPTORS, A. J. Daniels, Department of Pharmacology, Glaxo Wellcome Inc., Research Triangle Park, NC 27709.

The molecular determinants that govern neuropeptide Y binding to its receptors has been the subject of numerous studies. Research in this area has focused primarily on understanding which regions of NPY are essential for optimal affinity, biological activity, and receptor-subtype selectivity. There seems to be consensus that N- and C-terminal amino acids of NPY are essential for strong receptor interaction. Towards our ultimate goal of designing nonpeptide antagonists of NPY, we attempted to determine first the minimum structural requirements for receptor binding and activity. Our strategy focused on the examination of a series of C-terminal amidated short peptides containing aromatic and positively charged amino acids. A systematic structure-activity/activity study, led us to conclude that incorporation of the discontinuous epitope Try^{1}, Arg^{35} and Tyr^{36}-NH_2 into appropriately substituted pentapeptides, was sufficient to allow interaction with low micromolar affinity towards rat brain NPY receptors. Extension of these pentapeptides at the N-terminus, with the amino acids contained in NPY^{27-31}, generated an NPY agonist (YINLIYRLRY-NH_2) half as potent at NPY in inducing intracellular calcium increase in HEL cells and, in increasing mean arterial blood pressure in rats. Surprisingly, replacement of Leu^{4} by Pro in the above peptide agonist, afforded an NPY antagonist (YINPIYRLRY-NH_2) with nanomolar affinity for NPY receptors. Subsequent dimerization, via disulfide or lactam bridges, of a series of deca- and nonapeptides related to the above antagonist, afforded ligands with picomolar affinity for rat brain NPY receptors and potent antagonism for Y1 receptors in in Vitro and in in Vivo assays. It is of interest to note that while these dimers show an apparent high affinity for rat brain receptors (Y2 type mainly) they do not seem to interact with Y2 receptors in peripheral tissues such as the rat vas deferens. The latter suggests that central and peripheral Y2 receptors may represent different sub types of NPY receptors. The compounds described here represent a first generation of high affinity NPY receptor antagonists, which will help unveil the role of endogenous NPY in important physiological functions. In addition, these compounds may assist in the development of novel therapeutic agents to treat central as well as peripheral pathologies associated with an NPYergic transmission hyperactivity.

NEUROPEPTIDES IN LEARNING AND MEMORY PROCESSES, D. De Wied, Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands.

Neuropeptides are generated following gene expression in nerve cells and produced in large precursor molecules which are processed in biologically active peptides. The cascade of cell specific processes which express the genetic information determine the quantity and quality of the peptides synthesized. In this way neuropeptides with different, sometimes opposite and more selective properties are formed from the same precursor. Since the seventies, numerous peptides have been discovered in the brain. Time- and dose-dependent postlearning effects, modification of neuronal excitability, the presence of these neuropeptides and their receptors in brain structures in particular involved in information processing (cortex, hippocampus, amygdala, thalamus), greater effectiveness following intracranial treatment, and disturbances in learning and memory following immunoneutralization, gene- or receptor blockade are essential criteria for a neuropeptide to be involved in cognition per se. Vasopressin and oxytocin meet these criteria. Both neurohormones are converted in the brain into C-terminal fragments (AVP/OXT 4-9, 4-8) which are stronger acting and more selective on avoidance behavior. The active core of ACTH/MSH peptides on avoidance behavior is located in the sequences ACTH 4-7 and 7-16. Such peptides might be generated from ACTH/MSH in the brain. Structure activity studies with peptides related to ACTH/MSH led to the synthesis of a highly selective ACTH 4-9 analog (ORG 2766) devoid of inherent endocrine activities and a markedly increased potency on avoidance behavior. It appeared to be much more resistant towards enzymatic degradation and orally active. Most neuropeptides have context-specific effects. Vasopressin, for example has the most robust effect in fear-motivated behavioral test situations, while the effects of CCK, NPY and galanin are most prominent in food-motivated behavior, oxytocin, LHRH, and ACTH/MSH peptides in sexual components of learning (Kovács and De Wied, Pharmacol. Rev. 46, 269-291, 1994). A substantial number of studies with ACTH/MSH neuropeptides in particular with Org 2766, and with AVP, DDAVP and DGAVP in patients with cognitive disturbances as a result of head trauma, dementia e.o. revealed modest effects on cognition and social behavior in a number of subjects. Modest effects were also found in healthy volunteers.

NEUROPEPTIDE Y RECEPTOR SUBTYPES IN MAMMALIAN BRAIN: EVIDENCE FOR A BIBP3226 INSENSITIVE BINDING SITE LABELED WITH [^{125}I][LEU^{31},PRO^{34}]PYY, Y. Dumont and R. Quirion, Douglas Hospital Research Center and Dept. Psychiatry, McGill University, 6875 Boul. LaSalle, Verdun, Quebec, Canada, H4H 1R3.

Neuropeptide Y (NPY) is one of the most abundant peptides present in the mammalian brain. This peptide has been implicated in several CNS functions including feeding and anxiety-related behaviors. Thus far, at least four receptor subtypes have been characterized and designated as Y_1, Y_2, Y_3 and Y_4/PP_1 (The Physiologist, 38: A241-A261, 1995). Additionally, NPY binding sites have been detected in several species (Martel et al., Brain Res., 419: 403-407, 1987) and autoradioraphic studies have shown that the Y_1 and Y_2 subtypes are most abundant in the rat brain cortex and hippocampus, respectively (Dumont et al., J. Neurosci., 13: 73-86, 1993). Recently, comparative autoradiographic studies have revealed some difference in the distributional profile of the radiolabelled Y_1 agonist, [^{125}I][Leu^{31},Pro^{34}]PYY and antagonist, [^{3}H]BIBP3226 (Dumont et al., NeuroReport, In Press). In fact, membrane homogenates and autoradiographic binding studies revealed that the rat brain contains two populations of [^{125}I][Leu^{31},Pro^{34}]PYY/Y_1-like sites, one that is highly sensitive to the Y_1 antagonist, BIBP3226 while the other is not. The pharmacological profile of various analogues and fragments of NPY, PYY and PP to compete for [^{125}I][Leu^{31},Pro^{34}]PYY/BIBP3266-insensitive sites is similar but not identical to the newly cloned Y_4/PP_1 receptor subtype (hPP $\geq$ PYY $\geq$ NPY $\geq$ [Leu^{31},Pro^{34}]PYY >> rPP = NPY_{13-36} = PYY_{13-36} = aPP). Finally, we recently noted major species differences in the CNS distribution of [^{125}I][Leu^{31},Pro^{34}]PYY/Y_1-like, [^{125}I]PYY_{3-36}/Y_2-like and [^{125}I][Leu^{31},Pro^{34}]PYY/BIBP3226-insensitive binding sites. While the rat and mouse brains demonstrated rather similar distribution profile, guinea pig and primate brains expressed very low levels of the Y_2 and Y_1 subtype, respectively. Accordingly, care must be taken when extrapolating from one species to another on the characteristics of brain NPY receptor subtypes. This study was supported by the MRC of Canada.

SOMATOSTATIN: ALTERATIONS IN NEURODEGENERATIVE DISEASES AND POTENTIAL USEFULNESS IN THE CLINICS, J. Epelbaum, INSERM U.159, Centre Paul Broca, 2ter rue d'Alesia, 75014, Paris, FRANCE.

Since the early eighties, a number of studies convincingly demonstrated that somatostatin concentrations are reduced in the cortex and hippocampus of Alzheimer's disease (AD) patients. However, few studies investigated the causes of this relatively selective neuropeptidergic deficit in relation with the cholinergic impairment and neuropathology of the disease. We quantified these parameters in the brains of 12 women over 75 years of age which ranged from 27 to 1 in the Blessed test score (BTS). The results indicated that the somatostatinergic deficit was more regionally restricted to the frontal pole than the cholinergic ones, also evident in the parietal and temporal lobes. Both somatostatin and CholineAcetylTransferase (ChAT) impairments were related to the intellectual loss but somatostatin was never correlated to the neuropathology (neurofibrillary tangles and senile plaques); in contrast to ChAT which did correlate in the parietal and temporal lobes. Since somatostatin and ChAT were also correlated in the frontal and parietal lobes, these results suggested that cortical somatostatin-containing elements are only affected following the cholinergic ones. By semi-quantitative in situ hybridization, an overall reduction of labeled cell density was observed in patients with AD but a significantly lower level of expression of somatostatin mRNA/cell was only observed in the hippocampus. Thus, the ability of cortical cells to express somatostatin mRNA is partially preserved in AD. Furthermore, the degradation rate of somatostatin by peptidases is lowered in frontal and parietal cortices from AD patients, indicating a compensatory mechanism and ruling out the use of blockers of somatostatin degradation to pharmacologically increase the peptide concentrations. Finally, radioautographic measurements reveal only modest changes in the concentration of the high affinity SRIF A binding sites, indicating that somatostatin receptor-bearing elements are preserved in the disease. In summary, the somatostatinergic deficit in AD is not a primary event but is likely to intervene in the dementia symptoms. Animal models, such as behaviorally impaired old rats or small primates (*microcebus murinus*) which reproduce partially Alzheimer's disease neuropathology and neurochemical deficits are of interest to assess the potential usefulness of somatostatin derived therapeutical approaches.

GROWTH HORMONE-RELEASING FACTOR AND ITS RECEPTORS IN AGING: RELEVANCE TO ALTERED HYPOTHALAMO-PITUITARY AXIS FUNCTIONS, P. Gaudreau, Neuroendocrinology Laboratory, Louis-Charles Simard Research Center, Notre-Dame Hospital and Department of Medicine, University of Montreal, Montreal, Canada

Growth hormone (GH) is released from the anterior pituitary into the circulation and contributes, either directly or through the action of insulin-like growth factor (IGF-I), to the maintenance of several tissue functions in adulthood. The pulsatile secretion of GH is regulated by two hypothalamic peptides, growth hormone-releasing factor (GRF) and somatostatin (SRIF). These peptides exert opposing actions on somatotroph cells by triggering specific G_s- and G_i-protein-coupled receptors. In middle age and old mammals, changes occur along the hypothalamo-pituitary GH axis, leading to a diminution of GH and IGF-I circulating levels. We have reported that a decrease in the number of high affinity pituitary GRF receptor binding sites appears early on in adult rats. This change precedes the diminution of GRF-induced GH secretion observed *in vivo* and *in vitro* in older animals. An increase in the number of low affinity GRF binding sites is seen thereafter, leading in old rats to a blunting of the high affinity binding sites and to an apparent reduction of the total number of sites. In addition, binding studies suggest that the GRF receptor develops a reduced ability to couple with its ligands and G-protein system. To further assess the importance of this receptor, we studied GRF binding in a rat model of successful aging. Interestingly, in rats submitted to a moderate calorie restriction from 8 to 18 months of age, pituitary GRF binding site parameters are similar in the old restricted and young control rats, while those of old *ad libidum* fed rats are deteriorated. Altogether, these results indicate that the pituitary GRF receptor status may play a significant role in the maintenance of the somatotroph function in aging. Molecular and immunological approaches will now be needed to elucidate some of the mechanisms involved in the modulation of GRF receptor sensitivity and functionality, in normal and successful aging.

NEW DEVELOPMENTS IN THE MOLECULAR PHARMACOLOGY OF NPY: THE SEARCH FOR THE FEEDING RECEPTOR, D. R. Gehlert, R. A. Gadski, D. Larhammar and S. Lyengar, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN and Department of Pharmacology, Uppsala University, Uppsala, Sweden

One of the most dramatic effects of centrally administered neuropeptide Y (NPY) and peptide YY (PYY) is a rapid induction of food intake. Through combination of both feeding and metabolic responses, chronically administered NPY induces obesity in rodents including increased white adipose tissue, decreased metabolism and insulin resistance. The pharmacology of the feeding response to NPY indicates a receptor with many of the properties of the Y1 receptor, however, the fragment NPY2-36 exhibits increased potency and efficacy when compared to the native peptide. In addition, centrally administered NPY reduces body temperature while NPY2-36 does not suggesting receptor heterogeneity in the feeding and metabolic responses to NPY. To help delineate the receptor family for the PP-fold peptide, we have employed molecular cloning techniques. The first member of the receptor family to be cloned was the Y1 receptor that exhibits relatively high affinity for the analog Pro^{34}-NPY while having lower affinity for the C-terminal fragments of NPY. Initially, this receptor was cloned as an "orphan" peptide receptor and later identified as Y1. Using homology cloning techniques, we recently cloned a human receptor that has limited homology to the Y1 receptor. This receptor exhibits very high affinity for pancreatic polypeptide (K_i~20 pM) with somewhat lower affinity for PYY (K_i~300pM) and NPY (K_i~3 nM). The rat ortholog of this clone exhibits poor amino acid conservation (75% identity) when compared to the human. rPP1 also has very high affinity for PP but substantially lower affinity for NPY and PYY. The third receptor we have cloned is a receptor with a pharmacology similar to the classically defined Y2 receptor. This was accomplished using an expression cloning technique with a cDNA library derived from human brain. The functional implications of the activation of these receptors subtypes *in vivo* will be discussed.

Our efforts have resulted in the cloning of the most established members of the PP-fold peptide family of receptors. However, none of these receptors possesses the complete pharmacology observed when PP-fold peptides are administered centrally in feeding studies. Further characterization of central NPY/PYY receptor subtypes by molecular cloning and the development and use of specific receptor antagonists will be necessary to identify the molecular target for this response.

NEUROPROTECTIVE STRATEGY FOR ALZHEIMER'S DISEASE: INTRANASAL ADMINISTRATION OF NEUROPEPTIDES, I. Gozes, A. Davidson, A. Bardea, M. Bechar, E. Giladi, S. Rubinraut*, M. Fridkin* and D. E. Brenneman**, Clinical Biochem., Sackler Med. School, Tel Aviv University, Isreal; *Organic Chemistry, Weizmann Inst., Rehovot, Isreal and **NICHD, NIH, Bethesda, MD 20892, USA

Vasoactive intestinal peptide (VIP) has been shown to be neuroprotective and play a significant role in the acquisition of learning and memory. A 100-fold more potent lipophilic analogue to VIP (J. Pharmacol. Exp. Therap. 273:161, 1995) has now been synthesized (stearyl-N1e^{17}-VIP), that exhibited neuroprotection in model systems related to Alzheimer's disease. Neuroprotection was observed at subpicomolar concentrations against the beta amyloid neurotoxicity *in vitro*. Intranasal delivery prevented impairments in spatial learning and memory associated with cholinergic blockade [achieved in rats by blockade with ethyl choline aziridium (AF64A)](Proc. Natl. Acad. Sci. USA 93:427, 1996). To further evaluate the breadth of neuroprotection in Alzheimer's-related models, mice deficient in apolipoprotein E (ApoE, Cell 71, 343, 1992) were tested as well. ApoE has been associated with neurotrophic function following injury as well as with the pathology of Alzheimer's disease. Our results indicated that ApoE-deficient mice exhibited retardation in the acquisition of developmental milestones. Furthermore, brains from these mice displayed a marked reduction in the mRNA encoding for VIP. Daily treatment with stearyl-N1e^{17}-VIP resulted in a significant improvement in the acquisition of the behavioral milestones, even earlier than observed in control animals. The neuroprotective activity of VIP is mediated via glial cells and secretion of growth factors from glial cell that in turn control neuronal survival (J. Cell Biol. 104:1603, 1987). A new neuroprotective protein (14,000 Daltons, pI: 8.3 $\pm$ 0.25) secreted by astroglial cells in the presence of VIP was recently isolated and named activity-dependent neurotrophic factor (ADNF), as it protected neurons from death associated with electrical blockade. This protein exhibited structural similarity to hsp60 and neuroprotective activity in unprecedented femtomolar concentrations (J. Clin. Invest. In press). A 14-amino acids peptide derived from this novel growth factor exhibited neuroprotection in the above-mentioned disease models. Our studies suggest a new therapeutic strategy for Alzheimer's disease with lipophilic VIP-related molecules. Furthermore, these studies identify a potent neuroprotective hsp-like glial protein and peptide that may mediate VIP functions and provide a basis for developing novel preventative treatments for Alzheimer's and other neurodegenerative diseases.

REGULATION OF ALCOHOL INTAKE BY ANGIOTENSIN MECHANISMS, L. A. Grupp and S. Harding, Biobehavioral Research Department, Addiction Research Foundation of Ontario and Department of Pharmacology, University of Toronto

Angiotensin (ANG) II is an octapeptide present in the circulation, but also locally produced in peripheral tissue and in brain. ANG II causes a dose-dependent, antagonist reversible suppression of voluntary alcohol drinking mediated by the AT_1 receptor subtype. The inhibitory effect is specific to alcohol since water intake is enhanced and glucose intake is unaffected by this peptide. Since ANG II does not alter the absorption, distribution or metabolism of alcohol, its effect is not the result of a change in drug handling. Subfornical organ lesions attenuate the inhibitory effect, yet infusions of ANG II into the third or fourth ventricles enhance water intake but do not suppress alcohol consumption. These findings suggest a specificity in the brain circuits that mediate ANG II's effect and dissociate its effects on water intake from its effects on alcohol intake.

Many manipulations which reduce alcohol consumption are correlated with changes in ANG II activity. For example, the beta adrenergic agonist, isoproterenol, or the serotonin uptake inhibitor, fluoxetine, stimulate ANG II and reduce alcohol drinking. Low salt diets activate while salt-supplemented diets suppress ANG II activity and are correlated with enhanced or reduced alcohol consumption respectively. Genetically selected animal lines such as the NP rats or DBA mice which avoid alcohol show elevated ANG II levels compared to their high alcohol drinking P and C57/B1 counterparts which have lower levels of ANG II activity. Finally, hypertension is associated with chronic alcohol consumption and studies have shown that high ANG II hypertension is associated with low alcohol consumption while low ANG II hypertension is associated with high alcohol consumption.

These actions of ANG II suggest that it may represent a satiety signal and recent experiments indicate that it might produce satiety by engaging glucoregulatory mechanisms. In one study, injections of tolbutamide, a drug which releases insulin and blocks glycogenolysis, reduced alcohol intake when administered alone, yet when combined with ANG II, attenuated its suppressive effect on alcohol intake. In a second study, isoproterenol, an adrenergic agonist which stimulates glycogenolysis, also reduced alcohol intake. This effect was attenuated by indomethacin, a drug which is known to decrease glycogenolysis. In a final study, glucose injections attenuated morphine's ability to stimulate alcohol intake. Taken together these findings suggest that a satiety function mediated by glucoregulatory processes may be a broad explanatory concept for the inhibitory effect of ANG II and isoproterenol on alcohol intake and may be general enough to explain the suppressive effects of other peptides and transmitters on alcohol consumption.

INTERACTION BETWEEN NEUROTENSIN SYSTEMS AND THE STIMULANTS OF ABUSE, G. R. Hanson, Department Pharmacology & Toxicology, University of Utah, Salt Lake City, UT

The neuropeptide, neurotensin (NT) has been linked to extrapyramidal and limbic dopaminergic activity in what appears to be a reciprocating relationship. For example, the overall effects of NT on activated dopamine (DA) pathways is to antagonize the behavioral consequence; consequently, NT has been referred to as an endogenous neuroleptic. In addition, changes in DA receptor activity (i.e., D-1 or D-2 types) appears to alter the functional state of NT systems. Because of these interactions we tested the possibility that treatment with stimulants of abuse, such as methamphetamine (METH) and cocaine, altered NT pathways. The response by striatal and nucleus accumbens NT systems to both drugs was similar. Following treatment with high doses of either drug, the NT tissue content increased significantly by 12-hr, but returned to control by 24-48 hr. This effect was mediated principally by co-activation of D-1 and glutamate NMDA receptors. Studies which examined the mechanism were conducted with METH and revealed that large doses of this drug increased the expression of the NT/neuromedin gene. Using recently developed microdialysis techniques we observed that a low dose (0.5 mg/kg) of METH doubled striatal and accumbens NT release by D-2 mechanism, while a high METH dose (15 mg/kg) did not have an effect on release of this neuropeptide. The relevance of these findings will be discussed. (Research supported by grants DA 00869 and DA 09407)

FUNCTIONAL ROLE OF NEUROPEPTIDE Y IN THE CENTRAL NERVOUS SYSTEM, M. Heilig, Dept. of Clinical Neuroscience, Karolinska Institute, 17176 Stockholm, Sweden.

Neuropeptide Y (NPY), isolated by Tatemoto and Mutt in 1982, is the prototypical member of the NPY / pancreatic polypeptide (PP) family. The amino acid (a.a.) sequence of NPY is highly conserved, suggesting a physiological importance of this peptide. In the periphery, NPY is a well established co-transmitter of the sympathetic nervous system, and participates in peripheral cardiovascular control. In the central nervous system (CNS), NPY is present in widespread neuronal populations, i.e. ascending brain stem projections, intrahypothalamic pathways, and local interneurons within telencephalic areas. Two prominent CNS actions of NPY represent attractive targets for drug development: 1. Stimulation of food intake, in particular that of carbohydrates, mediated through hypothalamic mechanisms, and possibly controlled by the endogenous ob protein, leptin; 2. Reduction of experimental anxiety, mediated through the amygdaloid complex, and possibly representing an endogenous counterbalance to the major endogenous stress / fear signal, corticotropin-releasing hormone (CRH). Physiological and pharmacological actions of NPY are mediated through heterogeneous receptor population, suggesting a potential for precise targeting with novel drugs. Several receptor subtypes for NPY and related peptides have been cloned and characterized, i.e. those corresponding to the original Y1 / Y2 subdivision proposed on the basis of ligand affinity studies, in which the full a.a. sequence of NPY was required for activation of Y1 receptors, while Y2 receptors could also be activated by truncated C-terminal fragments. Peripheral pressor effects of NPY, as well as anti-anxiety actions of central NPY seem to be mediated through activation of Y1-type receptors. In hippocampus, actions of NPY on neuronal excitability possibly related to memory mechanisms are exerted though Y2 receptors. The receptor subtype mediating feeding effects of NPY is less clear, with some evidence to suggest the possibility that it differs both from conventional Y1 and Y2 receptors. Ongoing isolation and characterization of new NPY-receptor clones will provide information whether feeding and anxiety effects of central NPY-receptor activation can be separated, a likely prerequisite for development of therapeutically useful drugs targeting these functions.

ACIDIC FIBROBLAST GROWTH FACTOR (aFGF) STIMULATES AXONAL REGENERATION, R.G. Hill, J.M.A. Laird, G.S. Mason, K.A. Thomas, S. Boyce, F.D. Tattersall and R.J. Hargreaves, Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, UK.

aFGF is a member of the fibroblast growth factor family. It has mitogenic effects on many non-neuronal cells but also supports the survival, neurite growth and differentiation of neuronal cells 'in vitro'. It is present in peripheral nerves in high concentration and seems to originate from the cell bodies of sensory and motor nerve fibres.

The action of aFGF on peripheral nerve lesions, made under general anaesthesia, has been studied in adult rats. aFGF given topically to the site of a sciatic nerve crush injury or intravenously, stimulated the regeneration of motor axons and of myelinated sensory axons. Dose dependent increases in regeneration distance were seen after 3.6, 36 or 360 ng/day topically or after 3 or 10 μg/kg systemically. In a second series of experiments, the effects of aFGF were studied where peripheral neuropathy had been produced by loose ligation of one sciatic nerve. A dose-related effect of aFGF on thermal hyperalgesia was seen after intravenous administration of 0.03 to 3 μg/kg/day for 7 days.

The administration of heparin, a necessary factor for the activity of aFGF, had no effect on nerve regeneration when given alone.

We conclude that, as crush injury has little effect on the endoneurial tubes and supporting cells, the regenerative effects of aFGF are likely to be due to a direct acceleration of neuronal extension. It is possible that aFGF may be clinically useful in the treatment of peripheral neuropathy.

GRAFTS OF TROPHIC FACTOR SECRETING CELLS IN RODENTS AND NONHUMAN PRIMATE MODELS OF NEURODEGENERATIVE DISEASE, J.H. Kordower and D. Emerich, Department of Neurological Sciences and Research Center for Brain Repair, Rush Presbyterian Medical Center, Chicago, IL 60612.

This presentation will describe studies in which grafts of encapsulated cells genetically modified to secrete trophic factors prevent the degeneration of neurons which are selectively vulnerable in neurodegenerative disease. Six Rhesus aged monkeys (between 24 and 29 years old) received fornix transections followed by intraventricular transplants of polymer-encapsulated baby hamster kidney (BHK) fibroblasts which had (n=3) or had not (n=3) been genetically modified to secrete human nerve growth factor (hNGF). Monkeys receiving lesions and control grafts displayed extensive reductions in the number of ChAT (57-75%) and p75 NGFr-immunoreactive (53%) medial septal neurons ipsilateral to the lesion/implant. In contrast, monkeys receiving transplants of encapsulated hNGF-secreting cells display only a modest loss of ChAT-(0-36%) and p75 NGFr(7-22.4%) -immunoreactive septal neurons. All monkeys receiving the hNGF-secreting implants, but none receiving control implants, displayed robust sprouting of cholinergic fibers within the septum ipsilateral to the transplant. Just prior to sacrifice, the capsules were retrieved and determined to contain viable BHK cells releasing biologically relevant levels of hNGF. These data demonstrate that hNGF can provide trophic and tropic influences to aged primate basal forebrain supporting the contention that hNGF may prevent basal forebrain degeneration in Alzheimer's disease.

In the second series of experiments, polymer encapsulated cells genetically modified to secrete human NGF or CNTF were grafted into rodent models of Huntington's disease (HD). Unilateral striatal grafts of hNGF-secreting cells induced hypertrophy and significantly increased the optical density of intact ChAT-ir striatal neurons 1, 2, and 4 weeks post-transplantation relative to rats receiving identical grafts missing only the hNGF construct. NGF secreting grafts also induced hypertrophy of noncholinergic neuropeptide Y-ir striatal neurons. Lastly, a DHFR-based expression vector containing the human ciliary neurotrophic factor (hCNTF) was transfected into baby hamster fibroblast cells (BHK). Using an immunoisolatory polymeric device, encapsulated BHK-control cells and those secreting hCNTF (BHK-hCNTF) were transplanted unilaterally into the rat lateral ventricle. Seven days later, the same animals received unilateral injections of quinolinic acid into the ipsilateral striatum. Nissl stained sections demonstrated that BHK/hCNTF cells attenuated the extent of striatal damage produced by quinolinic acid. BHK-hCNTF grafted animals displayed only a 12% loss of ChAT-ir striatal neurons following intrastriatal quinolinic acid compared to an 81% loss of striatal ChAT-ir neurons seen in BHK-control rats. BHK-hCNTF grafted rats also displayed only a 20% loss of GAD-ir striatal neurons compared to a 72% loss of GAD-ir striatal neurons in BHK-control grafted animals. These results support the concepts that implantation of polymer-encapsulated hCNTF-releasing cells can be used to protect striatal neurons from excitotoxic damage and this strategy may ultimately prove relevant for the treatment of HD.

MELANOCORTIN MC3 AND MC4 RECEPTOR EXPRESSION AND MELANOCORTIN EFFECTS IN FETAL AND EARLY POSTNATAL RAT BRAIN, W. Lichtensteiger, V. Kistler-Heer, M.E. Lauber and M. Schlumpf, Institute of Pharmacology, University of Zürich, CH-8057 Zürich, Switzerland.

Melanocortins are supposed to be involved in ontogeny and regeneration of nervous tissue. We recently reported on region- and stage-specific developmental patterns of binding sites for [^{125}I]Nle, D-Phe-α-MSH (^{125}I-NDP) in rat central nervous system, cranial nerve ganglia and sympathetic ganglia, with a number of regions exhibiting marked transient peaks of receptor expression (Lichtensteiger et al., Dev. Brain Res. 91:93, 1996). NDP binds to the main melanocortin receptors found in brain, i.e., the MC3 and MC4 receptor, with comparable affinity. In subsequent in situ hybridization experiments using ^{33}P-labeled oligonucleotides, we so far have detected MC3 mRNA only in postnatal rat brain. Ventromedial and arcuate hypothalamic nuclei, which exhibit a slow postnatal development of ^{125}I-NDP binding sites, contain MC3 mRNA during the first postnatal week. In contrast, MC4 mRNA is found in the majority of regions with high fetal and perinatal levels of ^{125}I-NDP binding. This suggests that the MC4 receptor may represent an important type of melanocortin receptor during prenatal ontogeny. Possible developmental actions of melanocortins were studied in cell cultures of striatum, a region exhibiting a high perinatal peak of ^{125}I-NDP binding with a subsequent drop to low levels in caudate-putamen and persistent elevated levels in accumbens and olfactory tubercle. Serum-free cultures of dissociated cells of striatum, combined with midbrain, were prepared from fetuses of time-pregnant rats during the rising phase of striatal MC4 receptor expression (gestational day 18). Preliminary data indicate a small to moderate increase of neurofilament content of the cultures (determined by ELISA) following short-term exposure to α-MSH and NDP, while the effect of ACTH is uncertain over an extended concentration range. GAP43 content remained unaffected. Additional developmental markers are being investigated. The results are suggestive of a developmental action of melanocortins at brain level, but require further analysis.

ROLE OF NEUROPEPTIDE FF IN OPIATE TOLERANCE AND DEPENDENCE, D. H. Malin, Univ. of Houston-Clear Lake, Houston, TX 77058.

Neuropeptide FF (FLFQPQRFamide, F-8-Famide, FMRFamide-like mammalian peptide, morphine-modulating octapeptide) occurs in certain locations involved in opiate analgesia and the precipitation of opiate abstinence syndrome. It potently modulates opiate actions, most commonly inhibiting them. This effect appears to be mediated by stereospecific NPFF receptors rather than opiate receptors. Chronic or continuous morphine exposure alters NPFF levels in brain tissue and CSF. ICV (third ventricle) injection of NPFF or peripheral injection of its lipophilic agonists precipitates opiate abstinence syndrome. Conversely, IgG from NPFF antiserum protects against naloxone-precipitated abstinence and temporarily reverses morphine tolerance on the tail flick test. Modification of the Rfamide C-terminal to R-amide results in analogs with NPFF antagonist activity, as indicated by the reversal of NPFF bioactivity. Such analogs reverse tolerance by ICV injection and reverse dependence (prevented abstinence) by both ICV and peripheral injection.

Recently, research has implicated nitric oxide, the calcium ion and the NMDA receptor in opiate tolerance and dependence. In this connection, it is interesting that NPFF is released by NMDA receptor stimulation, and its bioactivity appears to be dependent on calcium ions and nitric oxide synthase.

TARGETED MUTATION OF NEUROPEPTIDES REGULATING REPRODUCTION IN THE MOUSE, L. Muglia, C. Luedke, K-H. Jeong, L. Jacobson and J.A. Majzoub, Division of Endocrinology, Children's Hospital, Boston, MA 02115.

Inactivation of neuropeptide expression by homologous recombination in embryonic stem cells and subsequent production of homozygous deficient mice is a powerful tool for evaluation of in vivo gene function. We have exploited this methodology to assess the roles of corticotropin-releasing hormone (CRH) and oxytocin (OT) in modulation of mammalian reproduction. Previous studies by several groups employing foot shock, restraint, and food deprivation stress paradigms in combination with CRH antagonists have implicated CRH in the stress induced suppression of luteinizing hormone (LH) production. We have utilized CRH deficient mice to further evaluate the role of CRH during reproduction. CRH deficient mice exhibit impaired glucocorticoid production secondary to inadequate ACTH release during stressful and circadian stimuli, as well as low basal glucocorticoid production. Male and female CRH deficient mice are fertile despite adrenal insufficiency, indicating CRH is not required for reproduction. CRH deficient female mice, however, do not lactate normally and offspring fail to grow after the 2-3 days of life. Our recent studies have employed restraint stress, food deprivation, and adrenalectomy to elevate endogenous CRH in normal mice while evaluating differences in CRH deficient mice. Results of studies in male CRH deficient and wild type mice, with evaluation of LH, testosterone, mating efficiency, and fertility, will be presented. Additionally, we have made substantial progress in the production of an oxytocin deficient mouse line. The mouse oxytocin gene isolated from a 129/Sv genomic library was used to generate a null oxytocin vector for gene targeting. This construct encompassed 7.5 kb of oxytocin flanking sequences, while deleting the entire oxytocin coding region. The endogenous oxytocin allele has been efficiently targeted with this vector, with 10% of embryonic stem cell clones having undergone homologous recombination. High level male chimeras have been produced from several of these clones, and are currently being tested for germline transmission. This model will be used to explore the role of OT in sexual and maternal behavior, parturition, and fertility.

THE ROLE OF NEUROTENSIN IN NEUROENDOCRINE FUNCTIONS, W. Rostène, A. Nicot, M. Alexander[1], ER De Kloet[2], W. Rowe[3], A. Bérod. INSERM U.339, Hôpital St Antoine 75012 Paris, France; [1]Pharmacol Department Boston University, USA; [2]Sylvius Lab University Leiden The Netherlands; [3]Devl Neuroendo Lab, Hop. Douglas, Montreal, Canada

It has been known for a long time that neurotensin (NT) administered into the rat brain influences the secretion of several pituitary hormones. Concerning those involved in reproduction, depending on the model and its route of administration, NT induces various effects on LH release such as an increase in proestrus female, ovariectomized (OVX) or OVX-estrogen treated females following intracerebral administration in the preoptic area, or a decrease when injected intracerebroventricularly. Though anti-NT antibody injection consistently exerts opposite effects on LH and induces a decrease in FSH after intracerebroventricular injection in OVX rats, it is not clear whether NT can directly affect gonadotropin release. Indeed, all arguments agree for no direct effect of NT in the rat on the anterior pituitary suggesting that the effects of the peptide on that system take place in the brain. Some recent neuroanatomical data confirm such hypothesis. NT fibers and NT receptors are found in the mediobasal hypothalamus. Moreover, estrogens are able to increase the number of NTmRNA expressing neurons in the rostral preoptic area, a region containing neural circuitry essential for mediating the effect of ovarian steroids on GnRH secretion, and in the arcuate nucleus. Estrogen treatment also modulates NT receptors mainly located on VIP neurons in the suprachiasmatic nucleus. Since a large subset of GnRH neurons receives a direct projection from these VIP neurons, it may be one pathway through which hormonal and circadian inputs to GnRH neurons can be modulated by NT. We have recently developed, in collaboration with Sanofi Recherche, non-peptide receptor antagonists since the lack of specific tools has hampered studies on the possible involvement of endogenous NT in neurodocrine functions. Though not tested yet on LH and FSH release, intracerebral administration of NT receptor antagonists was reported to block NT-induced activation of the HPA axis in freely moving rats. When such antagonist as SR48692 is implanted into the PVN, it can attenuate the diurnal increase in plasma ACTH and corticosterone observed during the afternoon peak of the circadian rhythm. Such implants also result in a blunted response of ACTH and corticosterone to restraint or new environment stress. Finally, chronic treatment with SR48692 induces an increase in plasma prolactin levels as compared to non-treated male rats. In conclusion, the recent development of such new drugs may allow us to better understand the physiological roles of NT in neuroendocrine functions and in particular on the gonadal axis.

NEUROTENSIN AS A MODULATOR OF ALTERED CHOLINERGIC FUNCTION IN THE BRAIN, W. Rowe*, P. Lapchak, D. Araujo, A. Beaudet and R. Quirion, Departments of Psychiatry, Neurology and Neurosurgery, Douglas Hospital, Res. Center, McGill University, Montreal, Canada, H4H 1R3

Cognitive deficits are often associated with an impairment of cholinergic function. However, functional changes in the activity of other neurotransmitter systems such as neurotensin (NT) may also underlie certain cognitive behaviors most likely, via its actions on the cholinergic system. The distributional profile of NT and acetylcholine (Ach) in the rat and human brain suggest that a great deal of anatomical overlap exists between these two neurotransmitter systems. Acetylcholinesterase-positive neurons are in register with NT receptors in both rat and man. Alterations in NT receptor density have been reported to be associated with the amnesia produced by lesions of basal forebrain neurons (Wenk et al., 1989, Behav. Neurosci., 103: 765-769). Thus, it would appear that NT may interact with certain cholinergic projections, either directly or indirectly to regulate its activity.

NT, at doses as high as 10 μM, had no effect on basal acetylcholine (Ach) release in rat striatal slices. However, it potentiated in a concentration-dependent manner, K^+-evoked Ach release. NT also modulated Ach release in both the frontal and parietal cortices suggesting a functional involvement with the cholinergic system in these regions as well. Further, these NT-induced effects in the cortical areas were abolished by prior lesioning of the basal forebrain cholinergic neurons.

The functional significance of a NT/cholinergic interaction may relate to the emergence of cognitive deficits in our population of aged (24 month old) Long-Evans rats. We have previously shown that aged cognitively impaired (AI) rats display decreases in cholinergic function as compared with our aged unimpaired (AU) and young (CTL) animals. Significant decreases in Ach levels and altered muscarinic M2 binding sites were found in both hippocampal and cortical areas of the AI animal. This correlated with decreases in [^{125}I]NT binding in the hippocampal formation, entorhinal cortex as well as the septum and hypothalamus in the AI animal. Alterations in [^{125}I]NT binding sites in the AI animals suggest the possible involvement of the NT-ergic system in the age-related cognitive deficits seen in this animal. The fact that changes in NT receptor density occurs in parallel with alterations in cholinergic activity in the AI animal suggests a possible interaction between these two systems in mediating this effect. This research is supported by a grant from the Medical Research Council of Canada.

NEUROTROPHIC FACTORS AND THEIR RECEPTORS IN CENTRAL CATECHOLAMINE SYSTEMS: EXPRESSION AND REGULATION, K. B. Seroogy, Department of Anatomy & Neurobiology, University of Kentucky College of Medicine, Lexington, KY 40536-0084

Both the nerve growth factor (NGF)-related and epidermal growth factor (EGF)-related families of polypeptide growth factors have been shown to support the survival, differentiation and maintenance of several neuronal populations within the central nervous system (CNS). In particular, members of both families are neurotrophic and neuroprotective for certain catecholamine neurons, including those of the dopaminergic ventral mesencephalon and the noradrenergic locus coeruleus. Work in this laboratory has been directed at determining the organization and regulation of these trophic factors and their receptors within catecholamine systems *in vivo*. Double-labeling studies indicate that adult rat midbrain dopaminergic neurons synthesize mRNAs for the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), as well as for their tyrosine kinase receptors *trk*B and *trk*C, respectively. These results support the notion that BDNF and NT-3 influence dopaminergic neurons via autocrine or paracrine mechanisms. Acute lesion of the nigrostriatal pathway with the catecholamine-specific neurotoxin 6-hydroxydopamine (6-OHDA) resulted in the transient increased expression of BDNF mRNA, and decreased expression of NT-3 mRNA, within neurons of the substantia nigra and ventral tegmental area. The early transient response of BDNF to toxic insult may reflect a potential neuroprotective role for the neurotrophin *in vivo*. Both *trk*B and *trk*C mRNAs are broadly distributed throughout the striatum. Long-term lesion of the nigrostriatal pathway resulted in the increased expression of *trk*B, but not *trk*C, mRNA in the caudate-putamen, indicating that midbrain dopaminergic afferents selectively regulate *trk*B mRNA levels in the striatum. The EGF receptor (EGF-R) ligand transforming growth factor-alpha (TGFα) is also expressed within the striatum, suggesting a typical target-derived trophic role for midbrain dopamine neurons, which express EGF-R mRNA. Lesion of the nigrostriatal pathway led to the decreased expression of TGFα mRNA in the caudate-putamen. Taken together, these data suggest that dopaminergic afferents normally inhibit the expression of *trk*B, and enhance the expression of TGFα, in the target striatum. Intriguing recent data indicate that a single injection of BDNF into the dopamine-depleted striatum, followed by a one-week survival period, normalizes the altered expression of both *trk*B and TGFα mRNAs within the striatum. The neurotrophins BDNF and NT-3 and their receptors are also expressed by noradrenergic locus coeruleus neurons. Studies to date indicate that the catecholamine-depleting agent reserpine, as well as traumatic injury to the brain or spinal cord, induce a substantial regulation of BDNF mRNA, and downregulation of NT-3 mRNA, within neurons of the locus coeruleus. Overall, the above data raise the possibility of select trophic factor involvement in neurological disorders associated with central catecholamine systems, including Parkinson's disease, schizophrenia, anxiety and depression. This work was supported in part by grants from the National Parkinson Foundation, American Parkinson Disease Association, Scottish Rite Schizophrenia Research Program, and NS35164.

EFFECTS OF OXYTOCIN ON THE NEUROADAPTIVE RESPONSES TO COCAINE, Z. Sarnyai, Laboratory of Neuroendocrinology and Laboratory of Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10021

Oxytocin's (OT) involvement in adaptive central nervous system processes has been demonstrated as an inhibitory, amnestic action on learning and memory in different paradigm. Learning, neuroadaptation and memory are likely to be involved in the neural events leading to drug abuse and dependence. Neurochemical processes influenced by OT, e.g. monoaminergic neurotransmission, are the major targets for the highly abused psychostimulant, cocaine. Therefore, we hypothesized that OT may modulate the neuroadaptation to cocaine. OT inhibited cocaine-induced stereotyped behavior, exploratory and locomotor activity in rats and mice and the rate of intravenous cocaine self-administration in rats. Behavioral tolerance to stereotyped behavior-inducing effects of cocaine was inhibited by OT. On the contrary, OT stimulated the development of behavioral sensitization to cocaine-induced locomotor hyperactivity in mice. ICV and IC (into limbic/basal forebrain sites) administration of an OT receptor antagonist inhibited the effects of peripherally administered OT on cocaine-induced stereotyped behavior. Microinjection of OT into the mesolimbic dopaminergic terminal regions (olfactory tubercle and nulceus accumbens) and ventral hippocampus attenuated the cocaine-induced stereotyped sniffing behavior and the development of cocaine tolerance, respectively. These data suggest that OT may act through basal forebrain/limbic target sites to modulate acute and chronic behavioral effects of cocaine. Acute and chronic cocaine administration resulted in an increase and a decrease in OT content in the hypothalamus and hippocampus, respectively, which may indicate a possible role of endogenous OT in cocaine-related neuroadaptive processes. OT selectively inhibited the behavioral effects of cocaine (a dopamine [DA] reuptake blocker) and low dose apomorphine (a presynaptic DA agonist), but not that of high dose apomorphine (a postsynaptic DA agonist) and amphetamine (a DA release stimulator). Cocaine-induced increase in DA utilization was attenuated by acute OT administration in the nucleus accumbens but not in the caudate nucleus. In summary, these results suggest that OT may act as a neuromodulator on the presynaptic DA terminals in the limbic/basal forebrain regions to regulate adaptive central nervous system processes related to cocaine abuse.

THE FIRST GENERATION OF ORALLY-ACTIVE NPY Y_1 RECEPTOR ANTAGONISTS: PHARMACOLOGICAL PROFILE OF SR 120819A, C. Serradeil-Le Gal, Sanofi Recherche, 195 route d'Espagne, 31036 Toulouse Cedex, France

Since NPY and related peptides, PYY and PP, exert their multiple effects through at least six receptor subtypes (Y_1, Y_2, Y_3, PP_1/Y_4, PYY-preferring and "appetite" receptors), there is a crucial need for specific tools to identify each entity and its functions. The last few years have shown significant progress in this field thanks to the cloning of receptors of the NPY family (Y_1, Y_2 and PP_1/Y_4) and to the design of the first potent and selective Y_1 receptor antagonists. We report here the discovery of the first generation of orally-active NPY Y_1 receptor antagonists, illustrated by SR 120819A. SR 120819A (1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[N-[4-(dimethylaminomethyl)-cis-cyclohexylmethyl]amidino]propionyl]-pyrrolidine) displays highly selective and competitive affinity for NPY Y_1 receptors from various species including man (K_i = 15 nM). Specific functional antagonism at Y_1 receptors has been demonstrated *in vitro* and *in vivo*, without observing any agonistic effects whatever the preparation used. Investigated in two Y_1 *in vitro* models, SR 120819A dose-dependently antagonized the inhibitory effect of NPY on adenylyl cyclase activity in the human neuroblastoma SK-N-MC cell line and counteracted the inhibitory effect of the Y_1 agonist, [Leu^{31},Pro^{34}]-NPY, in the rabbit *vas deferens* (pA_2 = 7.20 ± 0.07). *In vivo*, intravenous SR 120819A competitively blocked NPY-induced arterial blood pressure increase in pithed rats. Remarkably, both by intravenous (0.1 - 1 mg/kg) and by oral (1 - 10 mg/kg) routes, SR 120819A antagonized the [Leu^{31}, Pro^{34}]-NPY-induced hypertension in anaesthetized guinea-pigs with a long duration of action (> 4 H at 5 mg/kg p.o.). In addition, we demonstrated that SR 120819A constitutes a major tool for the characterization and localization of Y_1 receptors and potential subtypes in complex organs such as the rabbit kidney expressing mixed populations of NPY receptor sites. Thus, SR 120819A is the first powerful, selective, orally-effective NPY Y_1 antagonist yet described. This molecule represents the prototype of the first generation of *in vivo* active NPY Y_1 antagonists and is of relevance for understanding the pathophysiological role of NPY, Y_1 receptor functions and for developing compounds for therapeutic applications.

THE Y-RECEPTOR FAMILY: CLONED RECEPTOR SUBTYPES FOR NEUROPEPTIDE Y, PEPTIDE YY, AND PANCREATIC POLYPEPTIDE, M. W. Walker, C. Gerald, J. A. Bard, K. E. Smith, P. J.-J. Vaysse, E. L. Gustafson, M. M. Durkin, R. L. Weinshank and T. A. Branchek, Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652-1431 USA

The pancreatic polypeptide family includes neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP). These peptides function as neurotransmitters and hormones to regulate a variety of physiological functions including feeding, blood pressure, anxiety and nociception. Receptors for this peptide family are proposed to exist as distinct subtypes known as Y1, Y2, Y3, Y4 or PP1 and Y1-like. Subtype-selective ligands are predicted to be of therapeutic value, for example, in the treatment of obesity and pain. Our strategy has been to isolate human receptors as genomic or cDNA clones, to localize the receptors in human and animal model tissues, and to express homogeneous receptor populations as tools for drug design. The human Y1 receptor was first reported cloned in 1992. Our group and others reported the cloning of Y2 and Y4 subtypes from both human and rat in 1995. Y1, Y2 and Y4 receptors have a seven transmembrane spanning structure typical of G protein-coupled receptors. Y1 and Y2 mRNA share a broad distribution throughout the periphery and CNS, whereas Y4 mRNA is relatively more restricted. Receptor clones can be expressed transiently or stably in mammalian host cells for analysis in radioligand binding assays. Receptor clones can also be analyzed in stably transfected cells based on [Ca^{++}] mobilization or on the inhibition of forskolin-stimulated [cAMP], in which case the rank order of peptide potency mimics the rank order of affinity in radioligand binding assays. The putative Y3, distinguished by greater affinity for NPY and PYY, is reported to exist on rat colon smooth muscle. A putative Y1-like receptor was proposed after injection of NPY and related peptides into rat hypothalamus yielded in unique rank order for stimulation of feeding behavior: NPY_{2-36} > NPY, [Leu^{31},Pro^{34}]NPY > NPY_{13-36}. A putative PYY-preferring receptor in dog adipocytes and elsewhere is suggested by functional studies in which peptides were ranked for activity: PYY > NPY > NPY_{13-36} > [Leu^{31},Pro^{34}]NPY. A potentially unique receptor subtype in a human colonic cell line is suggested by peptide-dependent inhibition of ion transport in rank order: PYY > NPY > [Leu^{31},Pro^{34}]NPY > PP > NPY_{2-36}. We recently isolated a novel G protein-coupled receptor with homology to Y-type receptors. The pharmacology of this receptor will be discussed with reference to cloned or proposed subtypes, together with potential therapeutic applications.

ROLE OF EXTRAHYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR (CRF) IN DRUG AND ALCOHOL WITHDRAWAL SYNDROMES, F. Weiss, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA

CRF-containing neurons and CRF receptors in the central nucleus of the amygdala (CeA) are thought to have an important role in the mediation of behavioral and emotional responses to stress. For example, autonomic activation and anxiogenic responses associated with CRF administration can be mimicked by electrical stimulation of the CeA, whereas lesions of this nucleus or local administration of the CRF antagonist α-helical CRF(9-41) effectively reverse the anxiogenic behavioral actions of exogenous CRF. Symptoms of anxiety and negative affect are an integral part of drug and alcohol withdrawal syndromes. It is possible, therefore, that these withdrawal-associated symptoms are mediated by CRF neuronal mechanisms in the CeA. This hypothesis was explored by monitoring the release of CRF in the CeA of rats during ethanol, cocaine, cannabinoid, and opiate withdrawal, and by testing the effects of local administration of α-helical CRF(9-41) on behavioral signs of withdrawal. Removal of chronic ethanol treatment resulted in a progressive elevation of extracellular levels of CRF over a 12 h withdrawal period. Concomitant anxiogenic effects of ethanol withdrawal as measured on the elevated plus maze were effectively reversed by α-helical CRF(9-41). Increased CRF efflux in the CeA was also observed during cocaine withdrawal after 12 hrs of unlimited access to the drug. In addition, a gradual increase in CRF release was apparent already prior to withdrawal during the final hours of the 12 h self-administration episode, suggesting that the neuropeptide may play a role in the aversive subjective effects and/or the decrements in the reinforcing efficacy of cocaine associated with sustained continuous use (cocaine "bingeing"). Withdrawal from chronic cannabinoid treatment precipitated by a cannabinoid receptor antagonist strongly elevated extracellular CRF levels in the CeA with a time course that paralleled closely the behavioral manifestations of the cannabinoid abstinence syndrome. Finally, intra-CeA injections of α-helical CRF(9-41) reversed conditioned place aversion in morphine-dependent rats induced by administration of the opiate antagonist methylnaloxonium into the same site. Together, these findings provide support for an involvement of CRF mechanisms in the CeA in the regulation of behavioral and emotional consequences of drug and alcohol withdrawal and, consequently, in the motivational effects of withdrawal states. (Supported by NIDA DA08426 and NIAAA AA06420).

MECHANISMS OF REGULATION OF NEUROPEPTIDE EXPRESSION AFTER NEURONAL INJURY, R. E. Zigmond, Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106-4975

The pattern of peptide expression in many neurons remains plastic into adulthood. Interestingly, the most dramatic changes have been observed under conditions where these peptides may have neurotrophic actions, namely after axonal damage, when a neuron is deprived of its supply of target-derived trophic factors. This phenotypic plasticity has been examined primarily in the peripheral nervous system (in sympathetic, sensory and motor neurons); and the exact nature of the alterations in peptide expression differ between neuronal cell types. In normal animals, about 60% of the neurons in the superior cervical ganglion express neuropeptide Y (NPY), but few express galanin, vasoactive intestinal peptide (VIP), or substance P. However, within a few days after postganglionic nerve transection, these neurons decrease their expression of NPY and begin to express galanin, VIP, and substance P. Such changes are detected first at the mRNA level and later at the peptide level and can be localized to the principal neurons in the ganglion. Both leukemia inhibitory factor (LIF) and nerve growth factor (NGF) have been found to be involved in this phenotypic switch. LIF mRNA, though undetectable normally in peripheral ganglia and nerves, is induced in nonneuronal cells within 60 min after transection of the ganglion's main postganglionic trunks. When the effects of axotomy on peptide expression are examined in wild type and LIF-minus transgenic mice, the changes are substantially blocked in the knock out animals. Nerve transection also deprives the ganglion of target-derived NGF. Experiments in which intact animals were administered an NGF antiserum showed that loss of NGF *per se* increases expression of galanin and VIP and decreases expression of NPY. Furthermore, the increases in expression of galanin and VIP after axotomy are partially reversed *in vivo* by local application of NGF to the ganglion. Thus, the changes in peptide phenotype seen in axotomized sympathetic depend both on the induction of LIF and the removal of NGF. (Supported by NS12651 and 17512).

POSTER ABSTRACTS

1

IMPORTANCE OF THE FIRST C-TERMINAL RESIDUE OF DIBASIC CLEAVAGE SITES IN PRECURSOR PROTEOLYTIC PROCESSING. N. Brakch[1], M. Rholam[2] and P. Cohen[2]. [1]Division d' hypertension CHUV 1011 Lausanne Suisse. [2]Biochimie des Signaux Régulateurs Cellulaires et Moléculaire, Université Pierre et Marie Curie, Unité de Recherche Associée au CNRS, Paris France.

The selective pressure exerted on the evolution of hormone precursors and proproteins has led to the high conservation of basic residues as cleavage sites. However, examination of these sequences clearly indicate that whereas a number of the potential basic sites are indeed cleaved in vivo a significant percentage of these loci remain unprocessed. In an effort toward identification of other possible conserved residues in cleaved sites the amino acid sequences flanking 352 dibasic moieties (83 prohormones and pro-proteins) were examined. Frequency calculations on the occurrence of given residues at positions P_6 to P'_4 allowed us to delineate a number of features that might be in part responsible for discrimination between cleaved and uncleaved dibasic sites. Some amino acid occupy preferentially certain precursor subsites Met in P6 and P3, Asp and Ala in P'1, Pro in P6, Gly in P3 and P'2. In P'1 position the β-carbon branched side chain residues (Thr, Val, Leu, Ile) and Pro, Cys Met and Trp were either totally excluded or poorly represented, suggesting that they might be unfavourable to cleavage. The biological relevance of these observations was in vitro tested using both pro-oxcytocin convertase and Kex2 protease action on a series of pro-oxcytocin related synthetic substrates reproducing the Pro7-Leu15 sequence of the precursor in which the Ala13 residue (P'1 in the LysArg-Ala motif) was replaced by various amino acid residues. A good correlation was obtained on this model system indicating that P'1 residue of precursor dibasic processing sites is an important feature.

2

DEVAZEPIDE, A CCK-A RECEPTOR ANTAGONIST, ATTENUATES THE EXPRESSION OF SENSITIZATION TO THE BEHAVIOURAL ACTIVATING EFFECTS OF AMPHETAMINE. N.J. DeSousa[1], G.R. Wunderlich[1], and F.J. Vaccarino[1,2]. Departments of Psychology[1] and Psychiatry[2], University of Toronto, Toronto, Canada, M5S 1A1 and Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

Numerous lines of evidence suggest that dopamine (DA) release from terminals in the nucleus accumbens is a critical substrate for locomotor activation. The present studies investigated the possibility that endogenous cholecystokinin (CCK), acting via CCK-A receptors, modulates the expression of DA-mediated locomotion produced by either circadian (exp. 1) or pharmacological (exps. 2 and 3) manipulations. In exp. 1, animals received counterbalanced i.p. injections of the CCK-A receptor antagonist devazepide (0.001, 0.01, and 0.1 mg/kg) or vehicle during either their light- (LP) or dark-phase (DP), 30 min after which their activity was monitored. In exp. 2, activity was monitored in animals receiving counterbalanced i.p. injections of devazepide (0.1 mg/kg) or vehicle during their LP followed 30 min later by i.p. injection of either amphetamine (AMPH; 1.0 mg/kg) or saline. In exp. 3, animals were either sensitized to the locomotor activating effects of AMPH via injection of AMPH (1.5 mg/kg) in their home cage once per day for seven days or were given saline injections. Following a 10 day withdrawal period, AMPH-pretreated animals received an i.p. injection of devazepide (0.001, 0.01, or 0.1 mg/kg) or vehicle during their LP, followed 30 min later by i.p. AMPH challenge (0.75 mg/kg). Saline-pretreated controls were given vehicle injections followed by AMPH.

Results from exp. 1 revealed that while locomotor activity was greater during DP than LP testing, devazepide had no effect in either condition at any dose tested. Results from exp. 2 demonstrated that devazepide had no effect on locomotion induced by acute AMPH administration, and that this negative finding was independent of baseline exploration levels. Exp. 3 showed that AMPH-sensitized animals demonstrated a greater locomotor response to AMPH challenge than saline-pretreated controls, and that this augmented response was attenuated by devazepide at 0.1 mg/kg, but not at 0.001 or 0.01 mg/kg. Taken together, these data suggest that endogenous CCK, acting at the CCK-A receptor, modulates the expression of DA-mediated behaviour under conditions of elevated DAergic activity.

3

MOLECULAR BIOLOGY AND PHARMACOLOGY OF NPY/PYY/PP RECEPTORS: HOW MANY MORE SUBTYPES? C. Gerald, M.W. Walker, K.E. Smith, P.J.-J. Vaysse, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652-1431 USA.

Through activation of membrane bound receptors, neuropeptide Y (NPY) and related peptides participate in a diverse range of important physiological activities such as memory, pain, blood pressure, appetite, anxiety and circadian rhythm. Previous functional and binding data support the existence of multiple receptor subtypes including Y1, Y2, Y3, Y4 (PP), "atypical" Y1 and PYY preferring. To date, the rat, human and various other species homologs of the Y1, Y2 and Y4 receptor subtype cDNAs have been cloned and characterized. Protein sequence analysis reveals that the Y1, Y2 and Y4 receptors are members of the G protein-coupled superfamily. When compared to the Y1 subtype, the Y2 and Y4 receptors display an overall amino acid identity of only 31% and 42%, respectively (41% and 55% in the seven membrane spanning regions). Northern blot analysis reveals a broad distribution of the Y1 and Y2 mRNAs in human brain. Southern blot analysis is consistent with the human genome containing a single gene for each of the Y1, Y2 and Y4 receptors.

All three receptor subtypes have been reported to couple to inhibition of adenylate cyclase and increase in calcium mobilization. In addition, when injected in oocytes, the Y1 receptor has been shown to couple to the G protein-activated K^+ channel Girk1, and the Y2 subtype has been shown to inhibit Ca^{2+} influx through voltage-dependent calcium channels in neuronal cell lines.

Following in vivo feeding studies, the existence of an "atypical" Y1 receptor has been postulated by several groups. Subsequent to functional studies where the rank order of potency of the tested peptides did not fit any previously identified receptor subtype, a putative PYY preferring receptor has been proposed. The molecular biology and pharmacology of a newly isolated NPY receptor subtype will be discussed.

4

NPY-IMMUNOPOSITIVE STRUCTURES OF THE RAT AUTONOMIC GANGLIA DURING POSTNATAL ONTOGENESIS. V.V. Glinkina, L.A. Knyazeva, I.G. Charyeva, A.S. Pylaev. Dept. of Morphology, Russia State Medical University, Ostrovityanova str., 1, 117869, Moscow, Russia.

NPY is widely represented in the peripheral nervous system. Approximately one half of the monoaminergic neurons of sympathetic ganglia in various mammals contains NPY as a cotransmitter. NPY is visualized in nonadrenergic and noncholinergic neurons of the autonomic ganglia as well and can form any combinations with other neuropeptide (VIP, galanin, dinorphin, enkephalines, etc).

Among synaptic effects of NPY it may be noted on the one side inhibition of the noradrenaline release from presynaptic terminales and on the other side potentiation of the array of postsynaptic actions. Postnatal localization and dynamics of the density of NPY structures has not been examined. This study was intended to establish distribution of the NPY-positive structures in autonomic ganglia at various stages of the postnatal ontogenesis.

The following ganglia of the Wistar rats were studied: g. nodosum, lumbar ganglia of sympathetic trunk, main pelvic ganglion and intramural cardiac ganglia. The use of immunohistochemical method permits us to reveal three types of NPY-positive(+) structures: i- NPY+varicosities around the neurones; ii- thin NPY+ varicosities situated irregularly; iii- thick NPY+ fibres with the large number of varicosities. In addition, we found NPY-positive neurones. The pattern of the relationship and distribution of the NPY+ structures was an individual feature of each ganglion and age-dependent. Dynamics of the density of NPY+ structures was identical to all ganglia under study. It was characterized by a low level in newborn rats, marked elevation for first two postnatal weeks, and achievement of the definitive values toward the fourth week. Reduction of the number of the NPY+ structures was found in aged rats.

5

ANTISENSE MODULATION OF ANGIOTENSIN II AT_1 RECEPTOR PHYSIOLOGY IN ORGANOTYPIC CULTURES OF RAT BRAINSTEM. G.E. Gonye#, P. Hartig*, and J.S. Schwaber#. #Central Research Department, E.I. DuPont de Nemours & Co., Inc. and *Central Nervous System Diseases Research, DuPont Merck Pharmaceutical Company, Wilmington, DE 19880.

The primary afferent fibers mediating the baroreflex project onto neurons of the nucleus of the solitary tract (NTS), a nucleus containing receptors for a large variety of neuropeptides. Angiotensin II (ANG) causes well documented effects on blood pressure regulation, and AT_1 receptors in the medulla are expressed almost exclusively in the NTS. We have chosen the ANG/AT_1 system as a test system to validate a widely applicable approach to study neuropeptide-driven modulation of function at the cellular level. We have combined organotypic cultures and antisense knockdown to facilitate receptor level perturbation and subsequent physiological analysis. Knockdown of receptor expression mimics antagonists yet can circumvent the need for pharmaceutical reagents when none are available. Further, the required level of specificity demanded in receptor/ligand systems consisting of many closely related subtypes can be achieved. Organotypic brain culture has proven to be a functional intermediate between intact tissue and clonal cell lines preserving most of the organization and function of in vivo experiments. One common theme of receptor-mediated modulation, often uncovered by antisense knockdown, is a disparity of measured effects between the molecular and system levels. Small receptor binding deficits often result in much larger behavioral changes. Changes in AT_1 RNA and protein after culture in the presence of mismatch and antisense oligonucleotides are being determined by RT-PCR and in situ receptor autoradiography, respectively. Changes in functional coupling and cellular behavior are being determined by measuring c-fos induction and extracellularly recorded firing rate deltas after challenge with glutamate, ANG, or losartan, an AT_1-specific antagonist. The results of these ongoing investigations will be presented. Combining antisense knockdown with organotypic culture should prove widely applicable to ligand/receptor systems where appropriate pharmaceutical reagents are unavailable, and, ultimately, if the sole reagent available is the cDNA sequence.

6

PHARMACOLOGICAL CHARACTERISTICS OF BIOACTIVE PEPTIDOMIMETIC FOR NEUROPEPTIDE Y Y2 RECEPTOR USING THE TEMPLATE ASSEMBLED SYNTHETIC PROTEINS (TASP) CONCEPT. E. Grouzmann, Centre Hospitalier Universitaire Vaudois, Division d'Hypertension, M. Mutter, Institute de Chime Organique, Université de Lausanne, Suisse.

Template assembly of potentially bioactive peptide fragments offers a convenient tool for generating molecules with agonistic/antagonistic activity. We report on a TASP molecule (TASP-Y) in which the C-terminal tetrapeptide of NPY (NPY33-36) was covalently linked via its N-terminus to a tetrafunctional template (T). Binding of this peptide has been performed on Y1 and Y2 expressing cells. The results (Table below) show that TASP-Y binds selectively to a human cell line (LN319) that expresses Y2 receptors without binding to the SK-N-MC cells that exhibit Y1 binding sites.

Peptide	IC50 (nM) Y1 Receptor	IC50 (nM) Y2 Receptor
NPY1-36	0.5	0.085
NPY13-36	1000	0.126
NPY33-36	>10000	>10000
TASP-Y	4000	67.2
T	>10000	>10000

The in vitro functional activity of this molecule was investigated by measuring the intracellular free calcium concentration increase in response to NPY in LN319 cells. TASP-Y induces a shift to the right of the NPY response curve with a Ki at 43 nM. The results indicate that unlike NPY33-36, the TASP-Y analog is able to bind selectively to the Y2 receptor of NPY without any agonistic effect and is able to block the cellular response to a Y2 stimulation. Hence, TASP-Y is the first available potent and selective Y2 antagonist.

7

DOES BOMBESIN MEDIATE STRESS-RESPONSE THROUGH CRF RELEASE? EFFECTS OF BN ON BRAIN CRF, PLASMA ACTH, CORTICOSTERONE AND CATECHOLAMINES. P. Kent[1] and Z. Merali[1,2]. [1]School of Psychology and [2]Dept. of Pharmacology, University of Ottawa, Ontario, Canada.

A characteristic response to stress involves the coincident activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Activation of these systems results in specific endocrine and autonomic changes including increased plasma ACTH, corticosterone, norepinephrine, epinephrine and glucose levels. Current research indicates that many of these effects are mediated by corticotropin-releasing factor (CRF). It has already been established that central injections of the neuropeptide Bombesin (BN) elicits behaviors typically associated with increased emotionality and arousal such as increased grooming, shuttle escape deficits and suppression of food intake. Moreover, recent results from our laboratory have shown that stress alters levels of BN-like peptides in several brain regions. The objective of the current investigation was to elucidate the relationship between BN-like peptides and CRF. Results from our first experiment demonstrate that the central administration of BN (0.25 and 0.5 μg/3 μl), like CRF, caused a significant dose-dependent increase in plasma levels of ACTH, corticosterone, norepinephrine, epinephrine and glucose, 20 min post injection. Pretreatment with the CRF antagonist, αhCRF (10 μg/3 μl), significantly attenuated the BN-induced increases in plasma ACTH, norepinephrine, epinephrine and glucose, but had little or no effect on plasma corticosterone levels at this time point. The second experiment examined changes in CRF immunoreactivity in various brain regions 20 min following i.c.v. administration of BN (0.25 and 0.5 μg/3 μl). A significant reduction in CRF immunoreactivity was found in the amygdala, the anterior and ventromedial hypothalamic nuclei and the nucleus of the solitary tract. A trend towards a reduction in CRF immunoreactivity was observed in the median eminence, the lateral hypothalamus, the hippocampus and the prefrontal cortex, whereas no significant changes were found in the paraventricular or the dorsomedial nucleus of the hypothalamus. Taken together, these results suggest that 1) BN-like peptides may play a role in the mediation or modulation of stress response and 2) that some of these effects may be mediated via modulation of CRF release.

8

THE ACTIONS OF GALANIN AND M40 ON DELAYED NON-MATCHING TO POSITION IN ^{192}IgG-SAPORIN-LESIONED RATS. M.P. McDonald[1], G.L. Wenk[2], & J.N. Crawley[1]. [1]Section on Behavioral Neuropharmacology, ETB, NIMH, Bethesda, MD 20892, and [2]Division of Neural Systems, Memory and Aging, University of Arizona, Tuscon, AZ 85724.

Galanin is a 29-amino acid neuropeptide that coexists with acetylcholine (ACh) in the medial septum/diagonal band in the rat and inhibits acetylcholine release in the septohippocampal pathway. Galanin is overexpressed in the basal forebrain in Alzheimer's disease (AD). Galanin impairs performance on several rodent learning and memory tasks, including delayed non-matching to position (DNMTP). M40 (galanin[1-12]-Pro_3-$(Ala\text{-}Leu)_2$-Ala-NH_2), a peptidergic galanin receptor ligand, has previously been shown to antagonize galanin-induced impairment on DNMTP. The current experiments used a lesion model of AD to evaluate the actions of galanin and M40 on DNMTP when cholinergic transmission was reduced. Rats were injected with ^{192}IgG-saporin, an immunotoxin that selectively lesions cholinergic cells in the basal forebrain and produced a 54% reduction in hippocampal choline acetyltransferase in the present study. After recovery, rats were injected with galanin in the lateral ventricle and with M40 in the lateral ventricle or the ventral hippocampus. Galanin treatment significantly reduced choice accuracy in both the lesioned and sham groups. M40 along did not affect choice accuracy. These results suggest that blocking endogenous galanin is not sufficient alone to improve performance in lesioned rats, indicating the potential need for a combined cholinergic-galaninergic treatment strategy.

IMMOBILIZATION STRESS IS ASSOCIATED WITH INCREASED RELEASE OF CRF & BOMBESIN-LIKE PEPTIDES AT THE CENTRAL NUCLEUS OF THE AMYGDALA. J. McIntosh, Z. Merali. School of Psychology and Dept. of Pharmacology, University of Ottawa, Canada.

It is becoming increasingly evident that the amygdala, particularly the central nucleus (Ce), plays a key role in the emotional response to fearful/stressful stimuli. Neurons in the Ce are known to contain both bombesin (BN) and corticotrophin releasing factor (CRF). CRF is believed to mediate the stress induced coincident activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. However, underlying mechanisms causing CRF release are yet to be fully elucidated. Recent research in our lab has shown that acute stress increased BN-like immunoreactivity and that central administration of BN produces stress-like endocrine changes. Furthermore, pretreatment with a CRF receptor antagonist blocks the endocrine as well as some of the behavioral effects of BN. These results suggest that 1) BN-like peptides play a role in the mediation of the stress response and 2) that this response may be mediated through CRF release. The objective of the current study was to measure endogenous changes in release of BN and CRF in response to acute restraint stress in awake and freely moving animals using *in vivo* microdialysis. Probes were implanted at the Ce and four 30 min baseline samples were collected. Animals were then manually restrained for 20 min. Blood was drawn before and after restraint for ACTH and corticosterone levels. Perfusate samples were then collected every 30 min for up to 3 h. Animals were then exposed to a second session of restraint stress. Blood was again drawn and perfusate sampling continued for an additional 2.5 h. Results revealed a stress-related increase in both BN- and CRF-like immunoreactivity at the Ce. Following the first stress, there was an immediate increase in CRF which declined during the 2nd and 3rd samples. The second stressor produced an even greater rise in the CRF release. BN levels started to rise on the 2nd sample after the first stress, and continued to rise. This response was further enhanced by the 2nd stressor. These results appeared to be specific to the Ce as reflected by the lack of stress-induced changes at non-central amygdaloid sites. Plasma ACTH and corticosterone levels were significantly elevated following both periods of stress; however, this response was attenuated during the second stress suggesting habituation. These results demonstrate a stress related release of both CRH and BN-like peptides at the Ce of the amygdala which increases even more upon expose to a second stressful event. Unlike the stress-induced endocrine changes, these effects are more sustained and less prone to rapid adaptation. It is thus likely that these amygdaloid changes, particularly in the BN release, may mediate the more long-term effects of stress.

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NEONATAL CAPSAICIN TREATMENT BLOCKS FEEDING SUPPRESSION ELICITED BY SYSTEMICALLY BUT NOT CENTRALLY ADMINISTERED BOMBESIN. Z. Merali, D. Michaud. School of Psychology and Dept. of Pharmacology, University of Ottawa, Canada.

Capsaicin, a neurotoxin commonly found in chili peppers, has been shown to permanently degenerate some of the afferent neurons in neonatal rats. It is of interest that capsaicin treated rats display increased grooming and scratching behaviors like those seen following central injection of bombesin (BN). The purpose of this study was to determine whether neonatal capsaicin treatment altered response to BN in adulthood. On day two of life, capsaicin (50 mg/kg; s.c.) was administered to neonatal rats (n=20). The control group (n=20) received the same volume of vehicle. The weight gain was significantly lower in capsaicin-treated animals starting day 28 and this effect was independent of their activity level. The frequencies of scratching and grooming behaviors were significantly increased in the capsaicin group from day 21 to 42. Next we tested the response of both groups to systemically injected BN. Rats food deprived for 18 h were injected with various doses of BN (0, 4, 8, and 16 mg/kg; i.p.) in randomized order, and given access to food for a 4 h. A dose- and time-dependent suppression of food intake was observed in controls. However, BN failed to suppress food intake in capsaicin-treated animals. We next explored whether the response to centrally administered BN was also affected. Both controls (n=6) and capsaicin treated (n=6) groups responded to central BN (0, 0.1, 0.25, 0.5 mg; i.c.v.) in a dose- and time-dependent manner, and there were no significant group differences in terms of the satiety response. Thus the central efferent system mediating satiety effects of BN did not seem to be affected by neonatal capsaicin exposure. There are several controversial theories about whether systemic BN elicits its effects by endocrine, paracrine, or neural mechanisms. Our study clearly supports the notion that systemic BN mediates it's effects neuronally, through the capsaicin-sensitive myelinated A-delta and/or unmyelinated C-fibers of the primary afferent fibers.

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INHIBITION OF CRF-INDUCED CARDIOVASCULAR AND ENDOCRINE RESPONSES *IN VIVO* AND *IN VITRO* BY CP-154,526, A NOVEL NONPEPTIDE CRF RECEPTOR ANTAGONIST. R.M. Richter, M.J. Mulvany. Institute of Molecular Pharmacology, 10315 Berlin, Germany and Department of Pharmacology, Aarhus University, 8000 Aarhus C., Denmark.

Corticotropin-releasing factor (CRF), a 41-amino acid hypothalamic peptide, plays a critical role in activating the hypothalamo-pituitary-adrenal (HPA) axis by controlling the release of ACTH from the anterior pituitary and may act as a neurotransmitter in both brain and periphery. CRF evokes prominent stress responses as well as marked cardiovascular effects. With the recognition that CRF also plays a role in the pathophysiology of various stress-related disorders, CRF antagonists may have therapeutic application. Recently, a novel nonpeptide CRF receptor antagonist, CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-ethyl-amine. Pfizer), was developed.

In the present study we evaluated the antagonist potency of CP-154,526 in blocking central and peripheral mediated effects of CRF in five different bioassays *in vivo* and *in vitro*. The novel antagonist blocked in a dose-dependent (0.4 to 10 mg/kg, iv) and sustained fashion ($\approx$ 1 hour) centrally evoked (1 µg CRF, icv) cardiovascular responses (MAP and HR) in conscious rats. The ID_{50} was about 0.4 mg/kg for MAP and 0.8 mg/kg CP-154,526 for HR, respectively. In parallel, the CRF-induced increase in plasma ACTH and corticosterone levels was significantly attenuated after pretreatment with CP-154,526 (2 mg/kg, iv) (ACTH: by 80%, corticosterone: by 60% of the peak value).

In contrast, the ability of CP-154,526 (1 to 5 mg/kg, iv) to antagonize peripherally mediated cardiovascular responses (thought to be mediated through CRF_2-receptors) was weak, even with 50 µg/kg CRF, iv. Also, the ability of CP-154,526 to block the CRF-induced relaxation (1 to 100 nM; $EC_{50} \approx$ 10 nM) of pre-contracted rat mesenteric small arteries *in vitro* was low. Here CP-154,526 caused a marked shift in the concentration-relaxation curve only at a concentration of 3×10^{-6} M.

The results suggest that the nonpetide CRF receptor antagonist CP-154,526 blocks selectively biological activities mediated by the CRF_1 receptor identified in brain and pituitary.

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MOLECULAR CLONING AND EXPRESSION OF A cDNA ENCODING A PUTATIVE PEPTIDE RECEPTOR FROM THE RAT HYPOTHALAMUS. M.S. Statnick, N.G. Mayne, D.R. Gehlert, J.P. Burnett. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285-0424.

Neuropeptide Y (NPY) receptors represent a family of G-protein coupled receptors that have great molecular diversity. To gain further insight into the molecular structure of NPY receptors, we have attempted to clone new members of this receptor family. Using degenerate primers to conserved sequences of the neuropeptide Y1 receptor and the polymerase chain reaction (PCR), we amplified and cloned a cDNA fragment from rat genomic DNA. A PCR generated single-stranded ^{32}P-labeled probe of this cDNA was used to screen a rat hypothalamus cDNA library. Three clones were isolated and sequenced. Analysis of these sequences indicated an incomplete 5' coding sequence. Using asymmetric PCR in the rat hypothalamus cDNA library, we cloned the remaining 5' sequence and reconstructed the full coding sequence of the cDNA. The ~2.5 kb "composite" clone encodes a 306 amino acid protein that has seven hydrophobic domains. The cloned cDNA has highest homology to NPY, somatostatin and opioid receptors. Southern blot analysis of rat genomic DNA suggests that this receptor is encoded by a single gene. By Northern blot analysis a single ~6-kb transcript was identified in the rat brain. No mRNA was detected in any of the peripheral tissues examined. Analysis by RT-PCR showed that the receptor is expressed in many areas of the brain and pituitary. Using *in situ* hybridization, the highest levels of expression were localized to the anterior and intermediate lobes of the pituitary, reticular thalamic nucleus, paraventricular nucleus of the hypothalamus and granule cell layer of the cerebellum. Lower amounts of transcript were found in other brain areas. Following transient expression in AV12 cells, the receptor failed to bind NPY, somatostatin or opioid receptor ligands. Expression of an HSV-tagged version of the receptor in AV12 cells was detected in large amounts using fluorescence microscopy following transfection. Thus, it appears that cells transfected with this cDNA are capable of expressing the recombinant protein. We suggest that this cDNA encodes an orphan peptide receptor that may be involved in neuroendocrine regulation. The recent identification of an endogenous ligand that binds to an orphan opioid receptor offer approaches that may be taken to isolate the ligand for this receptor.

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